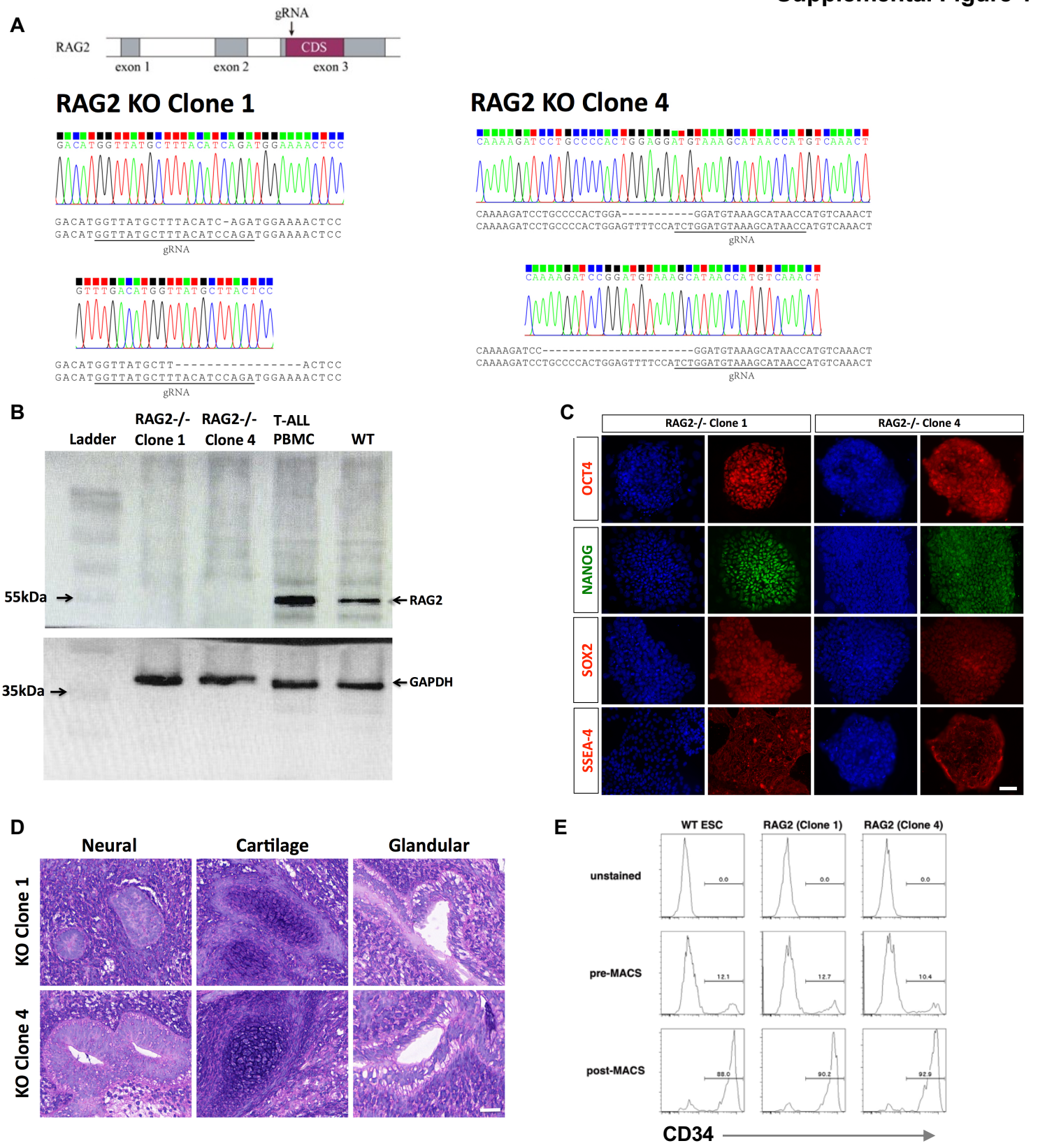
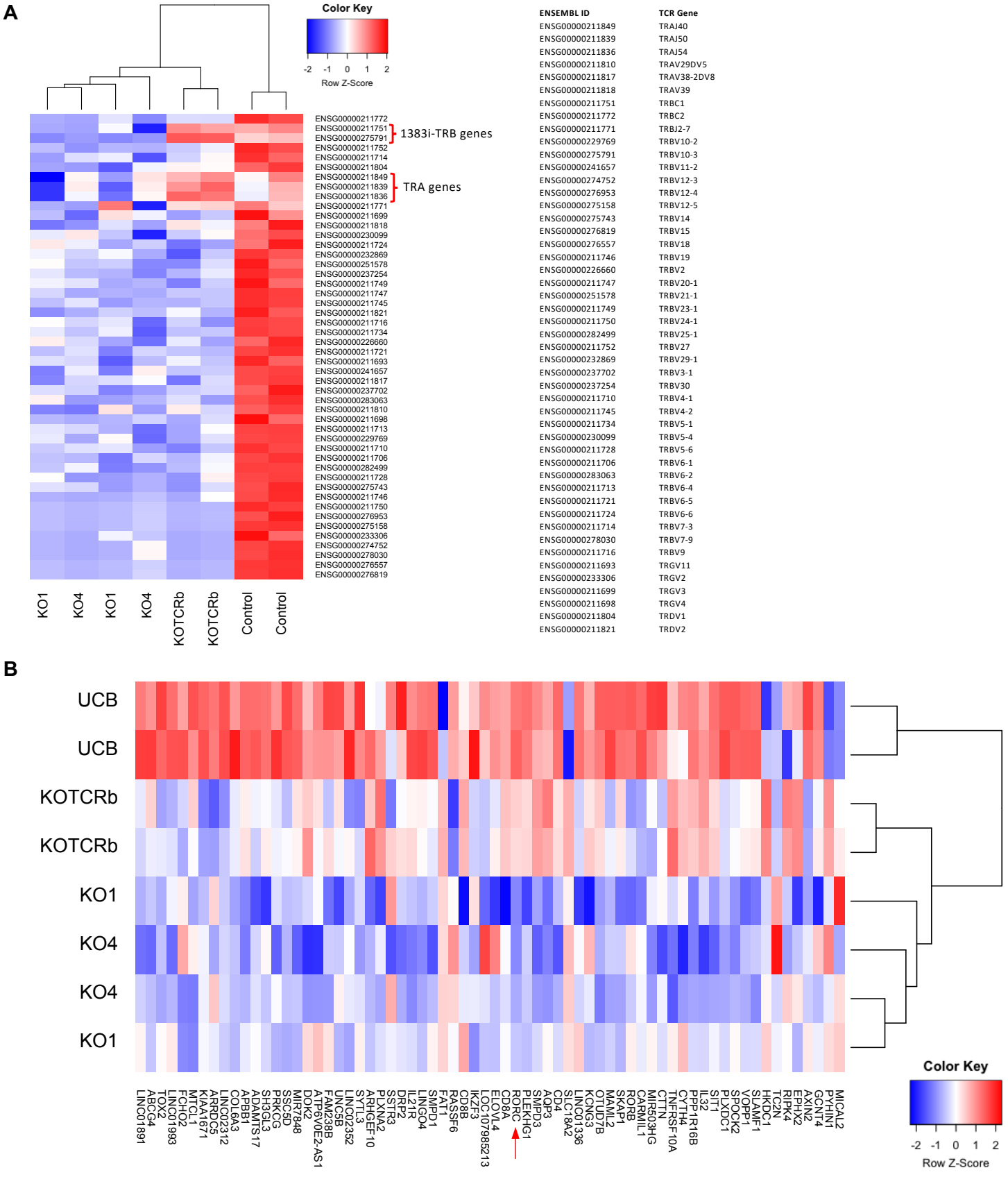


Supplemental Figure 1. Generation and characterization of RAG2-KO hESC lines. (A) Sanger Sequencing of genomic DNA of CRISPR/Cas9-targeted RAG2 of clones 1 and 4. The altered allele is depicted in chromatograph and sequence above it, while alignment of the altered and WT alleles are shown below. (B) Immunoblot for RAG2 protein expression of *in vitro*-derived T-lineage cells from RAG2-KO clones (1 and 4) and Control WT hESCs, or Control PBMCs obtained from a T-ALL patient. GAPDH serves as a loading control. (C) Immunofluorescence of RAG2-KO hESC clones for pluripotency markers. The scale bar corresponds to 100 µm. (D) Immunohistochemistry of RAG2-KO hESC clones against ectoderm (neural tissue), mesoderm (cartilage), and endoderm (glandular tissue) lineages of a teratoma formation assay. (E) $CD34^{+}$ hemogenic endothelial cell yield and enrichment after 8 days of embryoid body differentiation from Control WT, RAG2-KO-1, and RAG2-KO-4 hESCs. (n=3 of three independent experiments).

Supplemental Figure 2



Supplemental Figure 2. TCR gene expression and CD4⁺CD8⁺ signature gene expression. (A) Heatmap analysis of specific TCR genes expressed in Control WT DPs but absent in RAG2-KO1/4 DPs and TCRβ-transduced RAG2-KO1/4 DPs. (B) Expression of CD4⁺CD8⁺ signature genes (as determined by thymus DP signature genes shown in Supplementary Table 3) in RAG2-KO, TCRβ-transduced RAG2-KO, and umbilical cord blood (UCB) derived DPs. (n=2 for Control WT, RAG2-KO-1, RAG2-KO-4, and UCB, and n=1 for RAG2-KO-1 TCRβ-transduced and RAG2-KO-4 TCRβ-transduced of one independent experiment).

Supplemental Figure 3

Supplemental Figure 3. Analysis of proliferation and differentiation genes and biological pathways. (A) Heatmap analysis of differentially expressed genes in DPs from RAG2-KO1/4 (KO) and TCRβ-transduced RAG2-KO1/4 (KOTCRb) cells. (B) Names of gene ontology biological pathways that involve genes that are differentially up-regulated in Control WT DPs compared to RAG2-KO DPs. Highlighted are biological pathways that are relevant to cell survival and/or proliferation. Within the group of biological pathways that are involved in specific aspects of leukocyte regulation, the specific genes involved are also indicated.